10/009792

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 October 2001 (04.10.2001)

PCT

(10) International Publication Number
WO 01/73081 A1

(51) International Patent Classification[7]: C12N 15/70

(21) International Application Number: PCT/KR01/00549

(22) International Filing Date: 31 March 2001 (31.03.2001)

(25) Filing Language: Korean

(26) Publication Language: English

(30) Priority Data:
2000/17052 31 March 2000 (31.03.2000) KR

(71) Applicant *(for all designated States except US)*: **KOREA ADVANCED INSTITUTE OF SCIENCE AND TECHNOLOGY** [KR/KR]; 373-1, Kusong-dong, Yusong-gu, Taejon 305-701 (KR).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **LEE, Sang-Yup** [KR/KR]; 212-702 Expo Apartment, Chonmin-dong, Yusong-gu, Taejon 305-390 (KR). **JEONG, Ki-Jun** [KR/KR]; 102-411 Kaist Apartment, Kung-dong, Yusong-gu, Taejon 305-335 (KR).

(74) Agent: **LEE, Hau-Young**; Seowon Building 1675-1, 8th Floor, Seocho-dong, Seocho-gu, Seoul 137-070 (KR).

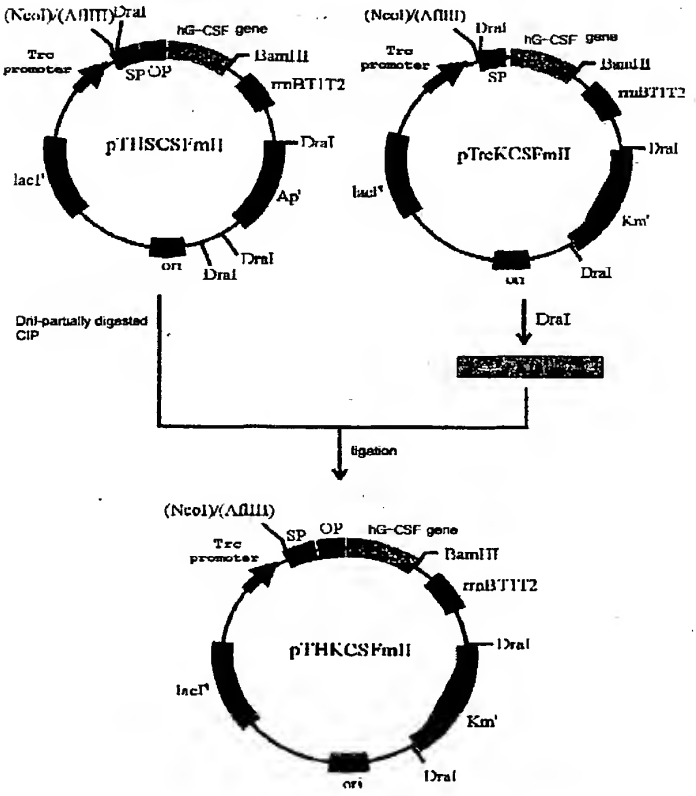
(81) Designated States *(national)*: CN, US.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

[Continued on next page]

(54) Title: ESCHERICHIA COLI STRAIN SECRETING HUMAN GRANULOCYTE COLONY STIMULATING FACTOR (G-CSF)



(57) Abstract: The present invention provides a recombinant plasmid vector comprising a kanamycin resistance gene, a promoter, an endoxylanase signal sequence, a nucleotide sequence coding for an oligopeptide consisting of 13 amino acids including 6 consecutive histidine residues, and a human granulocyte colony stimulating factor (hG-CSF) gene; an *E. coli* transformed with the said vector; and, a process for producing complete hG-CSF protein with high purity from the protein pool secreted by the said microorganism. In accordance with the invention, the hG-CSF protein can be prepared with high purity through rather simple process facilitating secretion of large amount of hG-CSF fusion protein into the periplasm, which does not require complicated processes such as solubilization and subsequent refolding required for isolation of the hG-CSF protein produced in cytoplasm as insoluble inclusion bodies by conventional techniques, thus, the hG-CSF protein can be widely used as an active ingredient in the development of supplementary agents for anticancer therapy.

WO 01/73081 A1